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Effects of ultraviolet radiation on the microbiological, physicochemical, and sensory properties of Rangpur lime juice

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Abstract

Rangpur lime juice was subjected to ultraviolet (UV) radiation as a non-thermal technology and changes in/on quality characteristics (microbiological, physicochemical and sensorial) were determined in order to extend its shelf life. UV-C treated juice resulted in 2.33 log and 2 log reductions in aciduric microorganisms and yeast and mould count, respectively. After UV-C treatment, there were no significant changes ($p > 0.05$) in pH, acidity and soluble solids; however antioxidant activity and the ascorbic acid content were affected by this process. The losses in ascorbic acid were 27.5 and 42% after UV-C treatment necessary to decrease 95 and 99% of the microbial load, respectively. Based on sensory analysis results, no significant differences were detected between fresh and UV-C treated juices. UV-C treatment extended the shelf life of fresh juice to 5 days during storage at 4°C.

Introduction

In recent years, consumer demand for minimally processed foods has increased rapidly. Generally, thermal processes are the most commonly used to inactivate microorganisms and enzymes, in order to extend the shelf life. However, these processes can produce adverse effects on sensory and nutritional quality of foods (Noci *et al.*, 2008).

In this context, a physical preservation method based on UV radiation is a useful alternative for solid and liquid foods. Ultraviolet treatment is performed at low temperatures and is classified as a non-thermal disinfection method (Guerrero-Beltrán and Barbosa-Cánovas, 2004), therefore this technologies can minimize loss of quality in terms of taste, aroma, color and nutritional value (Walkling-Ribeiro *et al*., 2008), compared to thermal processing. Other advantages associated with UV radiation is that no known toxic or significant nontoxic byproducts are formed during the treatment, certain organic contaminants can be removed, easy to use, the equipment is relatively inexpensive and it requires very little energy when compared to thermal pasteurization processes (Bintsis *et al.*, 2000; Keyser *et al.,* 2008). Besides, UV-C light (200-280 nm), which is part of the UV region (100-400 nm) has germicidal effect on microorganisms such as bacteria, yeasts, moulds and viruses (Caminiti *et al*., 2010). This technology meets a 5 log reduction of the pathogen microorganism(s) as a scientific criterion for pasteurization of juices (NACMCF, 2006). More than 5 log reductions of *E. coli* as a pertinent microorganism were reported in UV-C treated apple juice (Keyser *et al*. 2008; Franz *et al*., 2009). Also, up to 3 log reductions in natural flora of UV-C treated orange juice were revealed by Tran and Farid (2004) and Keyser *et al.* (2008). However, one of the main limitations in the efficacy of UV-C decontamination is the generally low UV penetration in fruit juices. For instance, the high absorbance of many natural substances in the UV range, the existence of soluble solids, large suspended particles or the high microbial populations reduce the capacity for UV-C penetration and lower considerably its efficacy (Bintsis *et al.*, 2000; Koutchma *et al*., 2007).

Rangpur lime (*Citrus limonia* Osbeck), a hybrid of lemon and mandarin, is native to India. The Rangpur lime is of horticultural importance primarily as a rootstock both in the Orient and South America and as an ornamental (Palacios, 2005). As a result of the high acidity of the fruit, Rangpur lime juice is used in South America in food and beverages as a replacement for lemon juice. Besides, Rangpur lime juice has an interest characteristic, an intense orange color as a consequence of high carotenoid contents (Chaves *et al.*, 2002).

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Most researchers suggests that the matrix (absorptivity, chemical composition and structural order) of the food itself, plays an important role in the damage caused by UV-C in the DNA of microorganisms, since that similar doses of UV-C have different effects on the growth of microbial species (Rivera-Pastrana *et al*., 2007). Therefore, it is important the evaluation of this technology on each particular product in order to define the optimal conditions of application and detect possible changes in the quality of the juice. In this work the effects of ultraviolet radiation treatment to prolong the shelf life of lime Rangpur juice, were evaluated on the microbiological, physicochemical and sensory characteristics of the juice.

Materials and Methods

Fruit material

Rangpur lime fruits (*Citrus limonia* Osbeck), were provided by the Experimental Station of the Faculty of Agricultural Sciences (UNNE) (Corrientes, Argentina). The juice was obtained by squeezing the mature fruits in an electric juicer (Arno, Facipress, Brazil) followed by filtration through a 2 mm^2 mesh sieve.

Ultraviolet light device

The Ultraviolet light irradiation device consisted of a germicidal lamp (PHILIPS / TUV 40 W with emission peak of 253.7 nm, voltage of 103 V and electric current of 0.43 A) placed horizontally in a chamber of stainless-steel (110 cm x 55 cm x 30 cm) with a cover protection for the operator. The excess of heat was dissipated by a ventilator installed on the back part and temperature recorded inside the chamber was never above 27°C.

Determination of UV absorptivity and turbidity of the juice

In order to establish the intensity of the UV lamp, we used the modified chemical method developed by Rahn (1997) which employs an iodide-iodate solution acting as an electron scavenger. The absorbance of 3.5 mL of a 0.6 mol/L KI solution was determined in 1-cm light path quartz cuvettes with a spectrophotometer (Metrolab 1700 V 2.03 model, Thailand) at 300 and 352 nm. Subsequently, the cuvette was placed horizontally, parallel to the UV lamp separated at different distances (10, 10.9, 13.5, 19, 19.5, 32, 33.5 and 42.5 cm). Then the absorbance was read at 352 nm. The lamp intensity was calculated using the equations proposed by Rahn (1997). Arbitrarily, we decided to work at a distance of 10 cm from the lamp with respect to the juice sample, so the value of current used was of 5.13 mw/cm^2 which was obtained by plotting the values obtained from the intensity of the lamp according to the distance measured. Turbidity was measured using a Parsec 520-G model turbidimeter (Argentina).

Determination of the UV lamp intensity

In order to establish the intensity of the UV lamp, we used a chemical method with some modifications that employs an iodide-iodate solution which acts as an electron scavenger (Rahn, 1997). The absorbance of 3.5 mL of 0.6 mol/L KI solution was determined in quartz cuvette, 1 cm path length, at 300 and 352 nm. Subsequently, the cuvette was placed horizontally, parallel to the UV lamp separated at different distances (10, 10.9, 13.5, 19, 19.5, 32, 33.5 and 42.5 cm). Then the absorbance was read at 352 nm. The lamp intensity was calculated using the equations proposed by Rahn (1997). Arbitrarily, we decided to work at a distance of 10 cm from the lamp with respect to juice samples, so the value of current used was of 5.13mw/cm^2 which was obtained by plotting the values obtained from the intensity of the lamp according to the distance measured.

UV radiation treatment of the juice

Ultraviolet light treatment was applied to Rangpur lime juice, under batch conditions. Four mL aliquots were placed in sterile Petri plates (10 cm diameter). UV radiation presents different penetration capacities on different liquids systems. This feature depends on the absorptivity of the liquid; the content of soluble solids and particles in suspension (insoluble solids). Thus, in order to obtain successful results, the Rangpur lime juice should be exposed to radiation in a film of thin thickness. Considering any opaque fluid can be considered as transparent to UV light when its thickness is less than 1.6 mm (Tran and Farid, 2004), and that a film thickness of 1 mm of juice allows 90% of the light absorption (Sizer and Balasubramaniam, 1999), in this work we used 1 mm of film thickness, allowing an adequate transmission of UV radiation during the disinfection process.

The doses (mJ/cm^2) were calculated using the following mathematical expression (Equation 1):

$$\text{Dose} = I * t \tag{1}$$

where: I, intensity (mw/cm^2) and t, time (s).

The plates were placed at 10 cm from the lamp and the following UV doses were tested: 102.6, 154, 205.2, 307.8, 615.6, 923.4, 1231.2 and 1385 mJ/cm^2 (Table 1). Immediately, the treated juice was

Table 1. Relationship time-dose for the treatment applied to the juice

Time (s)	Intensity (mw/cm^2)	Doses (mJ/cm^2)
0	5.13	0
20	5.13	102.6
30	5.13	154.0
40	5.13	205.2
60	5.13	307.8
120	5.13	615.6
180	5.13	923.4
240	5.13	1231.2
270	5.13	1385.0
380	5.13	1949.4

transferred (in a sterile environment) to sterile glass bottles for microbiological determinations.

Microbiological analysis

Microbiological analysis included enumeration of total aerobic microorganisms (TA), aciduric microorganism (AM) and yeast and molds (YM) (Pascual Anderson and Calderón y Pascual 2000). Aerobic plate count was performed by pour plate method using Plate Count Agar (PCA, Britania), which was incubated at 35 ºC for 48 h. Enumeration of molds and yeast were performed on Potato Dextrose Agar medium (PDA, Britania), plates were incubated at 22ºC for 7 days. For the cultivation and enumeration of acid-tolerant spoilage microorganisms plates were incubated with Orange Serum Agar (OSA, Britania) at 30°C for 48 h.

Physical and chemical analysis

Total soluble solids were measured as °Brix using an refractometer (ATAGO model N 1, Tokyo, Japan) with a precision of ± 0.1 °Brix. Total titratable acidity was assessed by titration with 0.1N NaOH and expressed as g of citric acid/100 mL and the pH was measured with a pH meter (Metrohom pH/Ionometer 692, Herisau, Switzerland). The antioxidant activity was measured using the DPPH method, as described by de Ancos *et al.* (2002). The reaction mixture contained 0.1 mL of pure juice and 6 mL of DPPH (2,2-diphenyl-1-picrylhydrazyl radical) 7.61×10^{-5} M in methanol solution. After 20 min in dark, the absorbance of the resulting solution was recorded at 517 nm and results were expressed as mg of ascorbic acid/100 mL. The properties were measured in triplicate, before and after the treatment of ultraviolet radiation on the Rangpur lime juice, using the dose that kills 95% or 99% of the initial microbial load.

The determination of ascorbic acid was performed by high efficiency liquid chromatography (HPLC, Shimadzu LC-10A, Tokyo, Japan), after applying the following doses of UV treatment to juice: 0, 153.9, 307.8, 615.6, 1077.3, 1263 and 1949 mJ/cm^2. The contents of ascorbic acid were measured on an extract of phosphoric sample prepared with 50 mL of juice (Nísperos Carriedo *et al*., 1992) and before injection, sample were filtered with 0.45 µm membrane. Mobile phase acetonitrile: water (30:70 pH = 2.8), Supelcosil RP C18 column (Supelco, LC 18, Pennsylvania, USA) and UV-Visible detector (Shimadzu, SPD-10A, Tokyo, Japan) at 260 nm were used. Results were expressed as mg ascorbic acid/100 mL of juice. Two extracts were done for each determination and measurements were performed in triplicate.

Triangle test

Differences between the juice samples treated with UV radiation (1263 mJ/cm^2) and untreated were determined by the triangle test. The sample juices were prepared with 10% of juice and 10% of sugar. Panelists (n = 12) were served to each test person received three samples. One of the samples was different and the other two samples were duplicates. The panelists were asked to taste and to sign the different sample in the evaluation form. Right and wrong responses were counted and evaluated according to critical number of correct responses in triangle test (α = 0.01 importance level) (Meilgaard *et al*., 1999). Two triangle tests were performed by each panelist.

Consumer acceptance testing

Juice samples treated with UV radiation, stored at 4°C and were judged by a panel of 50 consumers, men and women, between 18 and 65 years. The sample juices were prepared like 2.6.1. In order to know the degree of overall acceptability, a hedonic scale (1 to 9) was used, where 9 = like extremely and 1 = dislike extremely (Meilgaard *et al*., 1999).

Shelf life

Untreated and UV treated Rangpur lime juices were stored in jars sterile glass at 4ºC during 2 weeks. The dose applied (1263 mJ/cm^2) was that necessary to decrease the microbial population in 95%. Sampling was done at about 5 day intervals and it was made a count of MA, YM and a sensory acceptability analysis.

Statistical analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey Test (α=0.05). Statistical analysis was performed using Infostat software (Di Rienzo et al., 2008).

Results and Discussion

Microorganisms present in juices

The chain of processing citrus fruits which aims to obtain derivatives, mainly juices, requires a precise knowledge of the different factors involved in safety for each foodstuff. The efficiency of the ultraviolet light treatment on the juice was evaluated through the quantification of aciduric microorganisms, molds and yeast. The low pH (2.5), that characterizes this juice, limits the growth of almost all bacteria (with the exception of some lactic such as *Leuconostoc* and *Lactobacillus*); selecting, on that way, the growth of molds and yeasts (Alzamora *et al.*, 1993; Tapia de Daza *et al.*, 1994).

The results of the microbiological analysis for Rangpur lime juice previous to UV radiation treatment showed negative counts for aerobic mesophiles microorganisms, and microbial growth in OSA and PDA culture mediums. Initially, untreated Rangpur lime juice had 3.18 log CFU/mL of AM count and 3.04 log CFU/mL of YM count.

Microbial inactivation

UV radiation presents different penetration capacities on different liquids systems. This feature depends on the absorptivity of the liquid; the content of soluble solids and particles in suspension (turbidity). The absorptivity coefficients and turbidity obtained for Ranpgur lime juice were 20.24 ± 1.1 cm^{-1} and 2400 ± 150 NTU, respectively. This last absorptivity coefficient is similar to that reported for apple juice (25.9 cm^{-1}) but it is lower than that reported for orange juice (47.9 cm^{-1}) (Koutchma *et al*., 2007). Moreover, the turbidity value that we determined for Rangpur lime juice is intermediate between orange juice (3759 NTU) and apple juice (972 NTU) (Koutchma *et al*., 2007).

Another factor influencing UV effectiveness as a microbicide is the thickness of the food layer exposed to radiation. Thus, in order to obtain successful results, the Rangpur lime juice should be exposed to radiation in a film of thin thickness. Considering any opaque fluid can be considered as transparent to UV light when its thickness is less than 1.6 mm (Tran and Farid, 2004), and that a film thickness of 1 mm of juice allows 90% of the light absorption (Sizer and Balasubramaniam, 1999), in this work we used 1 mm of film thickness.

The microbial count was carried out at different doses of UV radiation, from 0 to 1949 mJ/cm^2 (Table 1), considering that the intensity of the UV lamp was 5.13 mw/cm^2. Germicidal effect of UV treatment applied to Rangpur lime juice is shown in Figure 1. The inactivation of microorganisms increased linearly depending on the dose used. Kinetics of destruction of microorganisms followed the Chick law, which responds to a kinetic model of first order (Equation 2) (Guerrero-Beltrán and Barbosa-Cánovas, 2004):

$$\log\frac{N}{N_0} = -kD \qquad (2)$$

where: N_0, initial concentrations of microorganisms; N, concentration of microorganisms after UV treatment; k, constant that depends on the type of microorganism and environmental conditions and D, radiation dose (mJ/cm^2).

As a result of UV treatment applied, a reduction of 2.33 and 2 logarithmic cycles were obtained for AM and YM respectively. However, Keyser *et al.* (2008) achieved a reduction of less than 1 logarithmic cycle for YM in orange juice with a dose of 1638 mJ/cm^2.

The low microbiological load reduction could be attributed to the particles found in suspension that could act as barriers between UV radiation and microorganisms. A slope of - 0.00103 for YM with a coefficient of correlation of 0.99, was obtained from a detailed analysis of Figure 1, which is a lower value than that obtained for the AM (-0.0012) with a coefficient of correlation of 0.99. Considering these results, the kinetic of destruction for the different microorganisms studied can be expressed by the following equations (Equations 3 and 4):

$$N = N0.10^{-0,00103.D} \qquad (3)$$

$$N = N0.10^{-0,0012.D} \qquad (4)$$

where: N, number of viable microorganisms in juice; N_0, initial number of microorganisms in the juice prior to treatment and D, radiation dose (mJ/cm^2).

The decimal reduction constant (D_{10}) for any microorganism is defined as the UV dose necessary to inactivate 90% of viable microorganisms (Tran and Farid, 2004). The value of D_{10} for YM in Rangpur lime juice was calculated from the Equation 3, resulting in 971 mJ/cm^2, while through Equation 4 we obtained 833 mJ/cm^2 for AM. The value was higher than that obtained previously for orange juice: 119 ± 17 mJ/cm^2 (Tran and Farid, 2004).

This behavior could be explained considering we worked with juice films exposed to UV radiation in batch conditions. However, it is possible to enhance the efficiency of the UV radiation increasing the exposure of the liquid to the emission of the UV lamp using equipment capable of generating a turbulent flow, which also prevents the aggregates of

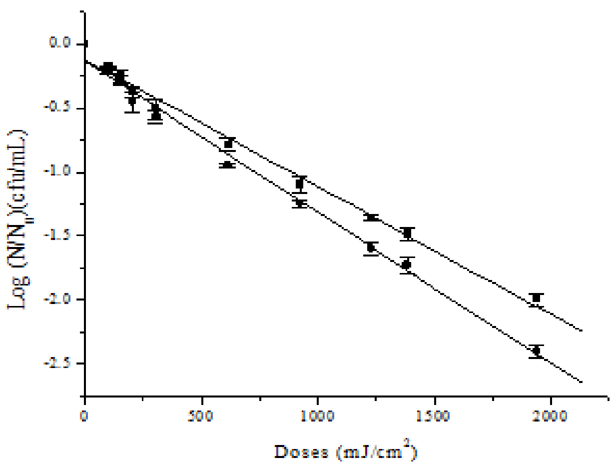

Figure 1. Log reduction of aciduric microorganisms (▲), molds and yeasts (●) after exposure to different doses of UV treatment

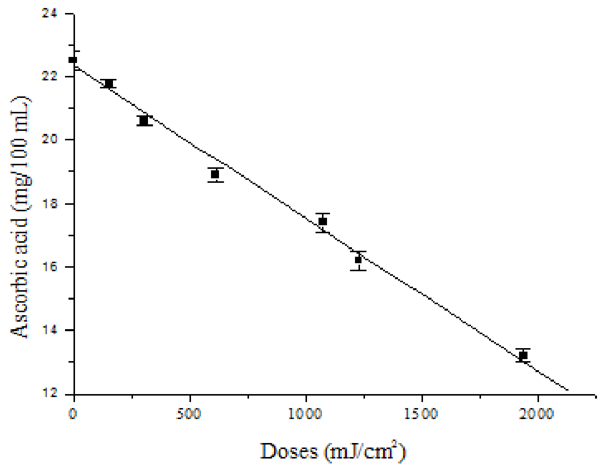

Figure 2. Ascorbic acid degradation in Rangpur lime juice exposed to different doses of ultraviolet radiation

microorganism and avoid the sedimentation of cells and other particles like fiber present in the Rangpur lime juice (Keyser *et al.*, 2008).

In our experimental conditions we identified the presence of different microorganisms compatible with *Aspergillus niger, Lactobacillus fermentum, Lactobacillus plantarum* and *Sacharomyces cerevisiae.* The existence of lactic bacteria among AM would explain the smaller D_{10} compared to that obtained for YM. That is, because the latter are more resistant to ultraviolet treatment, since that they are larger than bacteria (Guerrero-Beltrán and Barbosa-Cánovas, 2004), have fewer pyrimidine bases (mainly thiamine) and a thin wall cell (Tran and Farid, 2004). This may prevent the DNA replication in the normal way and disrupt gene functioning by creating new mutants. Although microorganisms have the capacity to repair this DNA damage, extensive damage may cross the limits of DNA repair mechanism leading to cell death. The resistance of microorganisms to UV is largely determined by their ability to repair such damage. In addition to the repair mechanisms, some organisms such as micrococci also synthesize protective pigments. Generally, the resistance to UV irradiation follows the pattern: Gram-negative <Gram-positive< yeast <bacterial spores < mold spores < viruses (Raso and Barbosa-Canovas, 2003).

Physicochemical properties

The doses used to evaluate the physical and chemical properties of Ranpur lime juice were those necessary to reduce 95% (1263 mJ/cm^2) and 99% (1949 mJ/cm^2) of YM count, who are the most resistant microorganisms present in the juice. The choice of these doses is in concordance with the Código Alimentario Argentino (1974) that accepts as a maximum limit the presence of 100 CFU/mL of YM in citrus juices. Thus, the D_{10} fails to meet this requirement since the initial load of YM was 1.1.103 CFU/mL.

The experimental values of pH, acidity, soluble solids and antioxidant activity, obtained before and after UV-C treatment of Rangpur lime juice, are shown in Table 2. The only property presenting significant differences ($p < 0.05$) was the antioxidant activity, which is mainly related to vitamin C degradation. Similar results were obtained by Uysal Pala and Kırca Toklucu (2011), Caminiti *et al.*, (2012) and Uysal Pala and Kırca Toklucu (2013) in pomegranate juice, apple juice and orange juice, respectively, after UV treatment.

Nutritional value in citrus is closely related to ascorbic acid (Iglesias *et al.* 2002), so its content is commonly used as a quality indicator for the orange juice shelf (Plaza *et al.*, 2006). Figure 2 shows the contents of ascorbic acid in Rangpur lime juice exposed to different UV doses. The equation that describes this behavior is: $y= 22.30492 – 0.00474^{*}D$, with a correlation coefficient of 0.99. Ultraviolet treatment produced a reduction of 27.5 and 42% when it was applied the doses that destroy the 95 and 99% of microorganisms.

Thus, the treatment applied to Rangpur lime juice to reduce the microbial load by 95% caused a reduction of 27.5% in the ascorbic acid content with a dose of 1263 mJ/cm^2. Moreover, Uysal Pala and Kırca Toklucu (2013) reported a 16.6% reduction of ascorbic acid in orange juice treated with a dose of 298.9 mJ/cm^2 of UV radiation.

Sensorial analysis

Triangle test as a discriminative sensory analysis determines whether or not a detectable difference exists between two juice samples. Panelists did not

Table 2. Physical and chemical properties of Rangpur lime juice measures applying different doses of UV treatment

Properties	UV treatment (Doses)		
	0 mJ/cm^2	1263 mJ/cm^2	1942 mJ/cm^2
pH	2.30±0.01^a	2.29±0.02^a	2.29±0.01^a
Acidity (g citric acid/100 mL)	5.98±0.15^a	6.09±0.02^a	6.12±0.25^a
Soluble solids (ºBrix)	7.8±0.2^a	8.0±0.1^a	8.0±0.2^a
Antioxidant activity (mg ascorbic acid/100 mL)	16.94±0.05^c	12.05±0.03^b	9.44±0.03^a

a,b,c; means with the same letters in the same properties do not differ significantly (p>0.05)

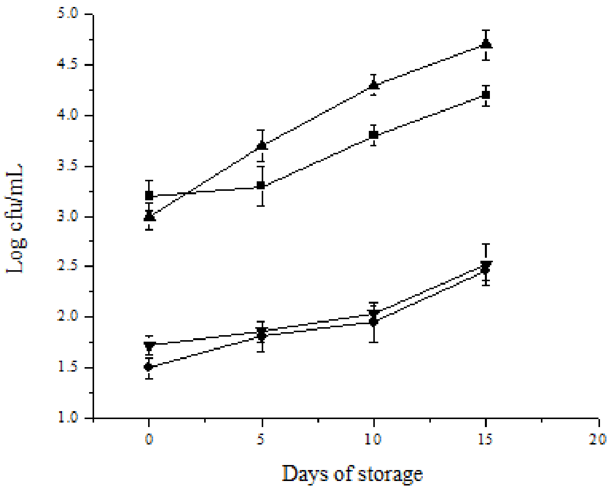

Figure 3. Changes in MA (■) and YM (●) counts of untreated Rangpur lime juice and MA (▲) and YM (▼) counts of UV treated Rangpur lime juice during storage at 4°C

found significant differences (α = 0.01) when they tested fresh Rangpur lime juice and that treated with the dose calculated to kill 95% of YM count. Donahue *et al.* (2004) and Uysal Pala and Kırca Toklucu (2013) also reported non- significant differences between untreated and UV-C treated (17.55 mJ/cm^2 dose) apple cider and UV-C treated (48.12 kJ/L dose) orange juice, respectively, after a triangle test.

Shelf life

Rangpur lime juices treated with ultraviolet radiation were stored in glass bottles (caramel color) to avoid the photoreactivation. This process occurs when microorganisms, damaged by UV-C treatment, absorb radiation of 330 nm (or higher) but repair alterations that have occurred at the DNA level, using protein factors (Guerrero-Beltrán and Barbosa-Cánovas, 2004).Consequently, from the physicochemical and sensory properties results, we decided to store at 4°C Rangpur lime juice treated with the dose required to kill 95% of microorganisms in order to prolong its shelf life.

The shelf life of fruit juices is limited by the growth of microorganisms, so they should be consumed fresh, generally within 24 hours (Song *et al.*, 2007). Logarithmic changes in MA and YM counts of untreated and UV-C treated Rangpur lime juice samples during storage at 4°C were shown in Figure 3. It can be observed, that evaluated microorganisms presented a similar behavior during the 15 days of storage. In these conditions, the YM and MA counts of treated juice had an increased 0.8 log and 0.9 log, respectively. On the other hand, Donahue *et al.* (2004) reported a 2.9 log rise in YM count of apple cider passed 4 times through a UV-C reactor after storage at 4 °C for 2 weeks.

Taking as a point of cut off the microbiological value accepted by the Código Alimentario Argentino (1974), the UV-C treatment increased in 5 days the shelf life of Rangpur juice. Similar results were obtained by Tran and Farid (2004) submitting Navel orange juice to an ultraviolet radiation (73.8 mJ/cm^2) treatment, and then storing it at 4°C. Thus, liking degrees of UV-C treated Rangpur lime juice samples storage for 0 and 5 days at 4°C were investigated using a consumer acceptability test. Both samples, in terms of overall characteristics were located the "extremely liking" part of the hedonic scale with a 8.22 ± 0.09 and 7.85 ± 0.20 scores.

Conclusion

UV-C treatment reduced the microbiological load of Rangpur lime juice maintains its nutritional and sensory attributes. UV-C processed juice had a longer shelf life under refrigerated conditions compared with untreated juice. This technology can be combined with other non-thermal preservation technologies with the aim to increase the log cycle reduction of microorganisms without affecting the sensory and nutritional quality.

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